REMARKS

Applicant has discovered polymorphisms in the human VCAM-1 gene, useful for determining allelic variation, and for diagnosing and treating VCAM-1 ligand-mediated diseases.

Claims 1-4, 6 and 7 are pending in the application. Claims 1, 4, 6, and 7 and the specification have been amended to replace or clarify references to EMBL ACCESSION NO:M92431 as SEQ ID NO:2. An amended sequence listing (paper copy and copy of verified statement are enclosed) is being filed concurrently with this response. Applicant submits that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification and the claims merely substitute the sequence identifier SEQ ID NO.:2 for references to the EMBL ACCESSION NO:M92431. The enclosed declaration from the inventor, John E. N. Morten, establishes that the sequence now designated SEQ ID NO:2 is identical to the sequence of EMBL ACCESSION NO:M92431 at the time the earliest priority application was filed (September 19, 1998). No new matter has been added.

Applicant notes the Examiner has withdrawn the rejection to claims 4, 6, and 7 for anticipation under 35 U.S.C. §102(b).

35 U.S.C. §101 and 35 U.S.C. §112, first paragraph

Claims 1-4, 6 and 7 remain rejected under 35 U.S.C. §101 and §112, first paragraph, because the invention allegedly "lacks a credible, substantial, specific or well-established utility" (Office Action, p. 2) and because "one skilled in the art would not know how to use the claimed invention" (Office Action, p. 4). This rejection is respectfully traversed.

The Examiner alleges that "the specification does not teach that an alteration at any of the specified nucleotide positions alters expression of VCAM-1," and further that "the specification has not clearly taught an association between the disclosed VCAM-1 polymorphisms and the occurrence of disease" (Office Action, p. 2).

VCAM-1 is a ligand for the α_4 integrin receptors and is involved in a variety of cellular processes. Its interaction with the $\alpha_4\beta_1$ integrin in particular has been implicated in T-cell proliferation, B-cell localization to germinal centers, hematopoietic progenitor cell localization in the bone marrow, angiogenesis, placental development, muscle development, and tumor cell

metastasis (Specification, p. 2). The variety and complexity of physiological process for which VCAM-1 plays a role demands an equally complex promoter to manage the expression of the gene in the appropriate tissues at the appropriate times and in the appropriate levels.

Expression of the VCAM-1 gene is low or absent in unactivated vascular endothelial cells, but is upregulated in response to inflammation causing expression of the protein on the surface of the cells. This upregulation is dependent on cytokines and is a hallmark of human inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, allergic asthma and atherosclerosis (Specification, p. 1). The upregulation of VCAM-1 in vascular endothelial cells during inflammation is regulated by transcriptional activation thought to involve the transcription factor nuclear factor-κB (NF-κB) (Specification, pp. 1-2).

In striking contrast to the low or absent basal expression of the VCAM-1 in endothelial cells, VCAM-1 is constitutively expressed on lymphoid dendritic cells, bone marrow fibroblasts, and certain macrophages (see Iademarco, *et al.*, Jour. Biol. Chem., 267:16323-16329, 1992). In addition, Iademarco *et al.* (Proc. Natl. Acad. Sci. USA, 90:3943-3947, 1993; copy enclosed) teach that a high basal expression of VCAM-1 in muscle cells is independent of cytokine activity. In fact, Iademarco *et al.*, 1992 (*supra*), report the identification of a silencer region at the 5′ end of the promoter, extending from nucleotide (nt) 540 to nt 1892 of the sequence corresponding to EMBL ACCESSION NO:M92431. This silencer region was shown by Iademarco *et al.*, 1992 (*supra*), to be responsible for the low basal expression level of VCAM-1 in endothelial cells. Five of the polymorphisms that are the subject of the present claims lie within the silencer region identified by Iademarco *et al.*, 1992 (*supra*).

Polymorphisms of the invention interrupt predicted consensus binding sites. Iademarco *et al.*, 1992 (*supra*), identified nine potential transcription factor binding sites in the silencer region of the promoter. One of these (at nt 1462-1470; AGTATG) is identical to a binding site for TEF-1 (Jiang *et al.* DNA Cell Biol., 19:507-14, 2000; see abstract enclosed), a tissue specific enhancer factor, and includes the site of the claimed polymorphism at nt 1467 (AGTA(T/C)G). In addition to the TEF-1 binding site, two potential binding sites for the ets proto-oncogene protein factors and three potential binding sites for octamer transcription factors were identified in the silencer region. Iademarco *et al.* (Proc. Natl. Acad. Sci. USA, 90:3943-

3947, 1993; copy enclosed) remarks that there are at least ten other sites in this region that show similarity to octamer binding sequences. In addition, and as acknowledged by the Examiner on page 2 of the Office Action, Applicant points out other specific known transcription factor binding sites that are created or interrupted by the polymorphisms.

The complexity of the VCAM-1 promoter is critical for its role in tissue-specific expression (the low basal expression of VCAM-1 in endothelial cells contrasts with the high basal level of expression in other cell types), and upregulation of the promoter is known to occur in response to the inflammation that is associated with the human inflammatory diseases mentioned above. The significant role of the promoter is evidence of a credible, specific, and substantial utility for the methods and compositions claimed in the instant application.

Identification of polymorphisms in the VCAM-1 gene of a human constitutes a credible and substantial utility. Applicant submits that the Examiner's remark that "the concept of analyzing a gene for a polymorphism to determine the status of an individual is considered to be a general use and is not considered to be a substantial, specific utility" (Office Action, p. 3) only applies when considering the analysis of any gene for a polymorphism in any individual. In the case of the instant application, Applicant claims a method for detecting a polymorphism in the promoter of the VCAM-1 gene specifically. In other words, the polymorphisms are in a gene whose overexpression is known to occur in the human inflammatory diseases mentioned above. In addition, the polymorphisms are in a region of the gene (the promoter) known to be a key regulator of expression levels. The MPEP §2107.01 states that "any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a 'substantial utility.'" In addition, the U.S. Patent and Trademark Office Utility Examination Guidelines (Fed. Reg. Vol. 66, No. 4 (January 5, 2001)) state, "An invention has a well-established utility...if a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention..." Certainly, a person of ordinary skill in the art would immediately appreciate the usefulness of detecting at least one of the cited nucleotide polymorphisms in the promoter of the VCAM-1 gene for the purpose of diagnosing an inflammatory disease or for determining a level of risk for an inflammatory disease in a human.

In view of the foregoing, Applicant respectfully requests that the rejections under 35 U.S.C. §101 and the related rejections under 35 U.S.C. §112, first paragraph be withdrawn.

35 U.S.C. §112, second paragraph

Claims 1-4, 6 and 7 remain rejected under 35 U.S.C. §112, second paragraph, as being indefinite over the recitation of "EMBL ACCESSION NO:M92431." Applicant has amended the claims and specification such that references to the EMBL ACCESSION NO:M92431 are replaced by references to the sequence identifier SEQ ID NO:2. An amended sequence listing distinctly claiming this subject matter is being submitted concurrently with this response. A declaration under 37 C.F.R. §1.132, from the inventor, John E. N. Morten, is also submitted herewith, wherein the inventor declares that the sequence referred to in the specification as EMBL ACCESSION NO:M92431 is identical to the sequence submitted as Exhibit A of the declaration and the sequence referred to as SEQ ID NO:2 in the amended specification and claims at the time the priority application was filed (September 19, 1998). As presently amended, claims 1, 4, 6, and 7 recite "SEQ ID NO:2" instead of "EMBL ACCESSION NO:M92431." This objection is therefore obviated, and Applicant respectfully requests that the rejection be withdrawn.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a Petition for Extension of Time for three months and a $930 check for the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 22 Jan 2003

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Version with markings to show changes made

In the specification:

Paragraph beginning at page 2, line 23 has been amended as follows:

Exon 1 of the VCAM-1 gene has been cloned and published as an EMBL Accession number: M92431 (2396 bp; hereafter referred to as SEQ ID NO:2) and all positions herein relate to the position therein unless stated otherwise or apparent from the context.

Paragraph beginning at page 3, line 8 has been amended as follows:

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in VCAM-1 in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions 278, 647, 707, 748, 829, and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2 [EMBL ACCESSION NO. M92431], and determining the status of the human by reference to polymorphism in the VCAM-1 gene.

Paragraph beginning at page 4, line 7 has been amended as follows:

In another aspect of the invention we provide a method for the diagnosis of VCAM-1 ligand-mediated disease, which method comprises:

i) obtaining sample nucleic acid from an individual,

ii) detecting the presence or absence of a variant nucleotide at one or more of positions 278, 647, 707, 748, 829, and 1467 (as defined by the position in SEQ ID NO:2[EMBL accession number M92431]), in the VCAM-1 gene, and

iii) determining the status of the individual by reference to polymorphism in the VCAM-1 gene.

Paragraph beginning at page 6, line 23 has been amended as follows:

In a further aspect, the diagnostic methods of the invention are used to assess the efficacy of therapeutic compounds in the treatment of VCAM-1 ligand mediated diseases such as

autoimmune, allergic and vascular inflammatory diseases. The polymorphisms identified in the present invention occur in the promoter region of the VCAM-1 gene. The changes are not expected to alter the amino acid sequence of VCAM-1, but several of the polymorphisms affect transcription sites within the promoter region and thus may affect the transcription of the VCAM-1 gene. For example the changing of the nucleotide at position 748 (as defined by the position in SEQ ID NO:2[EMBL ACCESSION NO. M92431]) from T to C results in the gain of an[a] E1a-F rev site and the loss of a TATA box.

Paragraph beginning at page 7, line 27 has been amended as follows:

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 278 in the promoter sequence;
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with G at position 647 in the promoter sequence;
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 707 in the promoter sequence;
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 748 in the promoter sequence;
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with A at position 829 in the promoter sequence;
the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 1467 in the promoter sequence;
or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one polymorphism.

Paragraph beginning at page 10, line 18 has been amended as follows:

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 647,

707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2 [EMBL ACCESSION NO. M92431].

Paragraph beginning at page 11, line 6 has been amended as follows:

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829, and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2[EMBL ACCESSION NO. M92431].

Paragraph beginning at page 11, line 25 has been amended as follows:

In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphism at 278 (as defined by the position in SEQ ID NO:2[EMBL ACCESSION NO. M92431]) because of its relatively high frequency (see below). The VCAM-1 gene has been mapped to chromosome 1p31-32 (Cybulsky et al Proc. Natl. Acad. Sci. USA **88**, 7859-7863, 1991).

Paragraph beginning at page 12, line 16 has been amended as follows:

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a VCAM-1 ligand antagonist drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in VCAM-1 gene in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 278, 647, 707, 748, 829, and 1467 (as defined by the position in SEQ ID NO:2[EMBL accession number M92431]), and determining the status of the human by reference to polymorphism in the VCAM-1 gene; and

ii) administering an effective amount of a VCAM-1 ligand antagonist .

Paragraph beginning at page 13, line 4 has been amended as follows:

According to another aspect of the present invention there is provided use of a VCAM-1 ligand antagonist drug in preparation of a medicament for treating a VCAM-1 ligand mediated

disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829, and 1467 (as defined by the position in SEQ ID NO:2[EMBL accession number M92431]).

Paragraph beginning at page 13, line 9 has been amended as follows:

According to another aspect of the present invention there is provided a pharmaceutical pack comprising VCAM-1 ligand antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829, and 1467 (as defined by the position in SEQ ID NO:2[EMBL accession number M92431]).

Sentence beginning at page 15, line 1 (in the Table legend) has been amended as follows:

[1]As defined by the position in SEQ ID NO:2 [EMBL ACCESSION NO. M92431]

Paragraph beginning at page 15, line 9 has been amended as follows:

Standard methodology can be used to detect the polymorphism at position[s] 647 (as defined by the position in SEQ ID NO:2[EMBL ACCESSION NO. M92431]) based on the materials set out below.

In the claims:

Claims 1, 4, 6, and 7 have been amended as follows:

1. (Amended) An assay for detecting a nucleotide polymorphism in the human VCAM-1 gene, which method comprises determining the sequence at one or more of positions 278, 647, 707, 748, 829, and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2[EMBL ACCESSION NO. M92431].

4. (Amended) An isolated and purified nucleic acid comprising any one of the following polymorphisms:

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 278 ;

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with G at position 647;

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 707;

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 748;

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with A at position 829;

the nucleic acid of SEQ ID NO:2[EMBL ACCESSION NO. M92431] with C at position 1467;

or a complementary strand thereof comprising at least one polymorphism or a fragment thereof of at least 20 bases comprising at least one polymorphism.

6. (Amended) An allele specific primer that specifically detects a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829, and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2[EMBL ACCESSION NO. M92431].

7. (Amended) An allele-specific oligonucleotide probe that specifically detects a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829, and 1467 in the VCAM-1 gene as defined by the positions in SEQ ID NO:2[EMBL ACCESSION NO. M92431].